

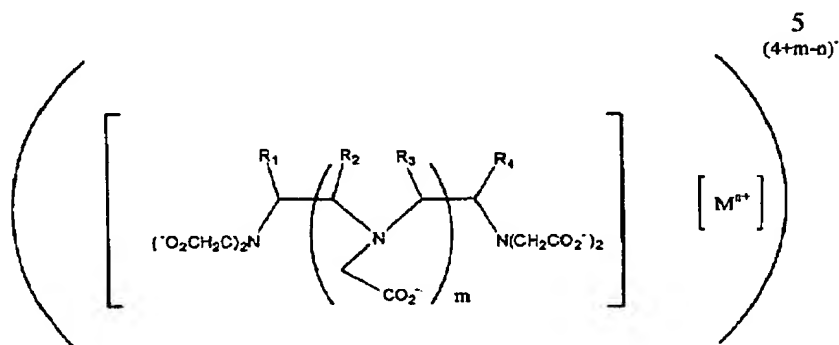
Page 2 of 26

15 March, 2005

Amendments to the Claims

I claim:

1. (Cancelled) A chelate-fluorophore tracer composition comprising:
a metal-chelated reagent having the formula



wherein m is 0 or 1; n is 1, 2, or 3; R_1 and R_2 are $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ or H, wherein R_1 must be H when R_2 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ and R_2 must be H when R_1 is

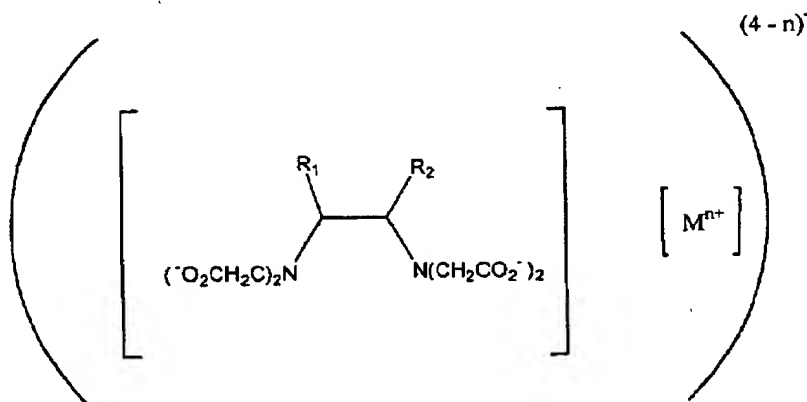
$p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$, and R_3 and R_4 are H, CH_3 , or are fused into a cyclohexyl ring

- 15 system; X is -HNC(S)NH- , -NHC(O)- or $\text{-NH-C}_3\text{N}_3\text{Cl-NH-}$; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium.
- 20

Page 3 of 26

15 March, 2005

2. (Cancelled) A chelate-fluorophore tracer composition comprising:
a metal-chelated reagent having the formula

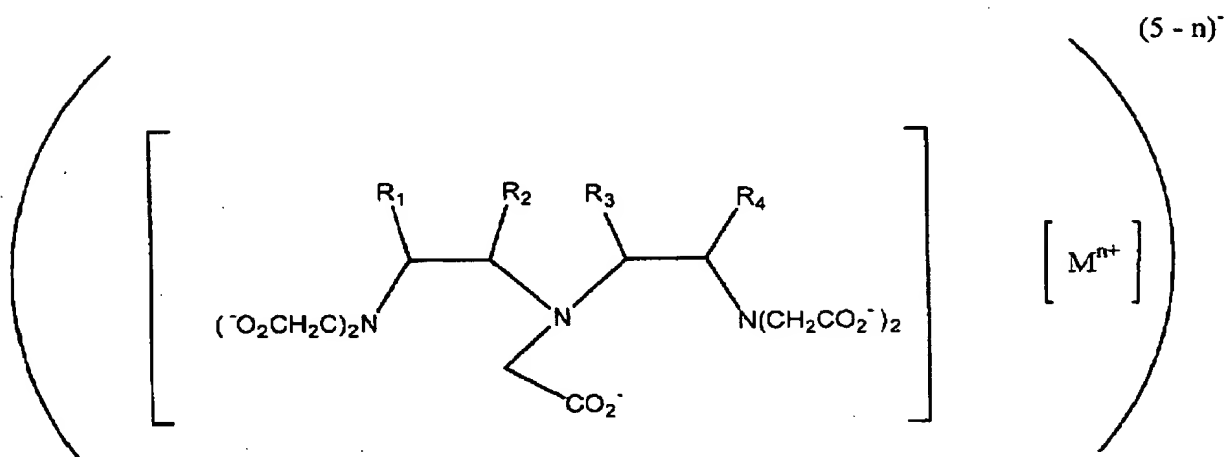


wherein n is 1, 2, or 3; R₁ is *p*-CH₂C₆H₄-X-Y, R₂ is H; X is -HNC(S)NH-, -NHC(O)- or -NH-C₃N₃Cl-NH-; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium.

Page 4 of 26

15 March, 2005

3. (Cancelled) A chelate-fluorophore tracer composition comprising:
a metal-chelated reagent having the formula



- 5 wherein n is 1, 2, or 3; R_1 and R_2 are $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ or H, wherein R_1 must be H when R_2 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ and R_2 must be H when R_1 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$; R_3 and R_4 are H, CH_3 , or are fused into a cyclohexyl ring system; X is -HNC(S)NH- , -NHC(O)- or $\text{-NH-C}_3\text{N}_3\text{Cl-NH-}$; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by
10 fluorescence polarization; and M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and
15 elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium.

Page 5 of 26

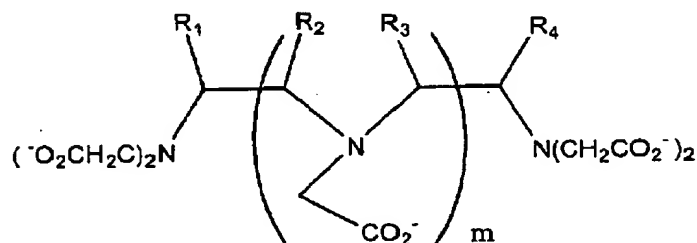
15 March, 2005

4. (Cancelled) The chelate-fluorophore tracer composition of Claim 2 or 3, further comprising the metal-chelated reagent wherein Y is selected from the group consisting of fluorescein derivatives, Texas red derivatives, rhodamine derivatives, coumarin derivatives, pyrene derivatives, naphthalene derivatives, and BODIPY dyes.
- 5
5. (Cancelled) The chelate-fluorophore tracer composition of Claim 2 or 3, further comprising the metal-chelated reagent wherein M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, and elements of Groups IIa, IIIa, IVa, Va, Via, VIIa, VIII Ia, and VIII Ib of the
- 10 Periodic Table of the Elements.
6. (Cancelled) The chelate-fluorophore tracer composition of Claim 2 or 3, further comprising the metal-chelated reagent wherein M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium,
- 15 cadmium, mercury, chromium, silver, antimony, barium, beryllium, thorium, zirconium, vanadium, nickel, molybdenum, manganese, zinc, cobalt, iron, and copper.
7. (Cancelled) A method for preparing a chelate-fluorophore tracer composition comprising:
- 20 a) adding a solution of a metal ion selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium, to an acidic

Page 6 of 26

15 March, 2005

solution of a fluorophore tracer composition comprising a chelating reagent having the formula



wherein m is 0 or 1, R_1 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ or H, R_2 is H or $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$, and R_3 and R_4 are H, CH_3 , or are fused into a ring system; X is -HNC(S)NH- , -NHC(O)- or $\text{-NH-C}_3\text{N}_3\text{Cl-NH-}$; and Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and

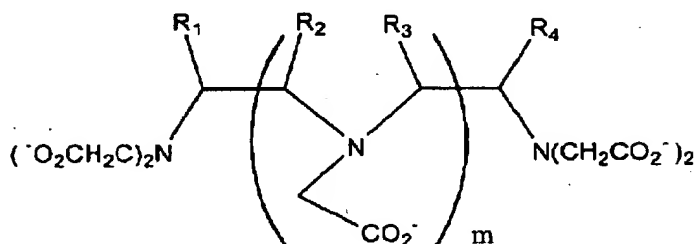
b) adjusting the pH of the resulting solution to about 7 or greater.

8. (Cancelled) A method for preparing a chelate-fluorophore tracer composition comprising:
 - a) adding a solution of a metal ion selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium, wherein the concentration of said metal ion in the solution is in the range from about 1 mM to about 20 mM, to a second, acidic solution of a fluorophore tracer composition

Page 7 of 26

15 March, 2005

comprising a chelating reagent having the formula



wherein m is 0 or 1, R_1 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ or H, R_2 is H or $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$, and R_3 and R_4 are H, CH_3 , or are fused into a ring system; X is -HNC(S)NH- , -NHC(O)- or $\text{-NH-C}_3\text{N}_3\text{Cl-NH-}$; and Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization wherein the concentration of said fluorophore tracer composition is in the range from about 1 mM to about 20 mM, the metal to tracer composition stoichiometry is about 1.0-1.1:1.0, and the pH of said acidic solution is about 2 or lower; and

b) adjusting the pH of the resulting solution to about 7 or greater.

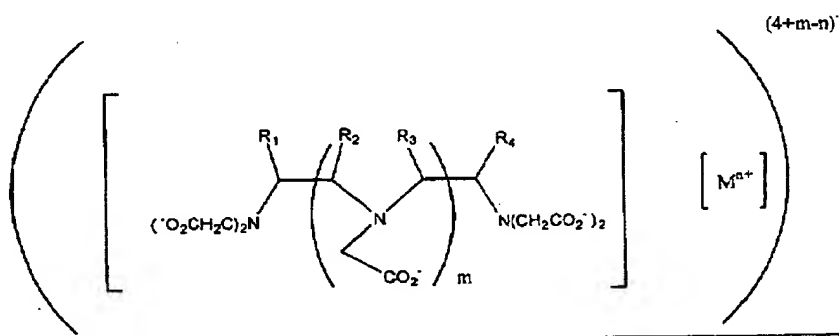
Page 8 of 26

15 March, 2005

9. (Amended Twice) A method for evaluating the metal selectivity of a macromolecular biological binding agent comprising:

- a) taking two aliquots having an equal volume combining each of a first series of serial dilutions of an aqueous solution thought to contain said biological binding agent and diluting each aliquot serially with equal volumes of with water to prepare a first and second series of serial volumetric dilutions of said aqueous solution;
- b) combining each dilution of the first series of serial volumetric dilutions of said aqueous solution with a fixed concentration of a first, target chelate-fluorophore tracer composition of Claim 1 comprising a metal-chelated reagent having the formula

10



15

wherein m is 0 or 1; n is 1, 2, or 3; R₁ and R₂ are p-CH₂C₆H₄-X-Y or H, wherein R₁ must be H when R₂ is p-CH₂C₆H₄-X-Y and R₂ must be H when R₁ is p-CH₂C₆H₄-X-

Y; R₃ and R₄ are H, CH₃, or are fused into a cyclohexyl ring system; X is

-HNC(S)NH-, -NHC(O)-, or -NH-C₃N₃Cl-NH-; Y is a fluorophore having a

fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody

binding at nanomolar concentrations by fluorescence polarization; and , wherein M is

the target metal, and measuring the polarization of the fluorescent signal obtained

20

when each resulting solution resulting from said combining is excited with plane-

Page 9 of 26

15 March, 2005

polarized light;

c) combining each dilution of a the second series of identical serial volumetric dilutions of said aqueous solution thought to contain the biological binding agent with a second, non-target chelate-fluorophore tracer composition comprising a metal-
5 chelated reagent having a formula identical to that of the chelate-fluorophore tracer composition of step b) with respect to m, n, R₁, R₂, R₃, R₄, X, and Y, but of Claim 1, wherein M is a non-target metal, said second tracer composition being present at the same concentration as the first tracer composition, and measuring the polarization of the fluorescent signal obtained when each resulting solution resulting from said
10 combining is excited with plane-polarized light;

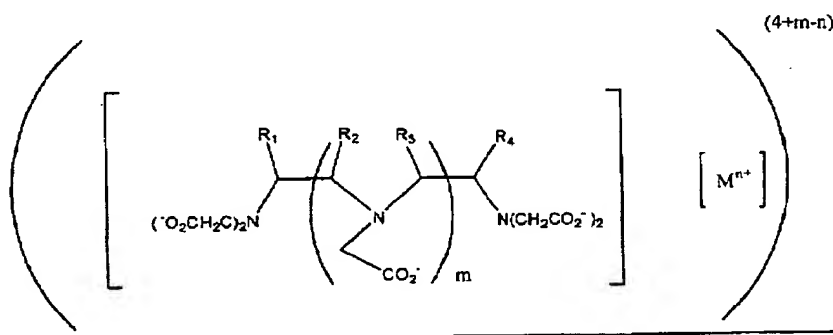
d) for each pair of solutions resulting from the combinations of steps b) and c), wherein the two solutions in the pair have the same extent of dilution, subtracting the measured value of the polarization of the fluorescent signal produced by each the solution containing that is obtained by combining the dilution of the second series of serial
15 volumetric dilutions of said aqueous solution with the non-target tracer composition of step b) c) from the measured value of the polarization of the fluorescent signal produced by the solution that is obtained by combining the dilution of the first series of serial volumetric dilutions of said aqueous solution, with the corresponding target tracer composition of step b) a) when measured at each sample dilution, whereby a
20 positive net value from the subtracting at any dilution less than that producing a baseline signal for the target tracer composition indicates the presence of a macromolecular biological binding agent that binds selectively to the target chelate-fluorophore composition and a zero or negative net value from the subtracting indicates no selectivity for said target chelate-fluorophore composition; and

Page 10 of 26

15 March, 2005

e) repeating steps b) ~~and e)~~ through d) for as many non-target metals as may be required to fully define the metal selectivity of the macromolecular binding agent according to its intended purpose.

- 5 10. (Amended Twice) A method for evaluating the metal selectivity of a polyclonal antibody response in a target chelate-immunized animal comprising:
- a) taking two aliquots having an equal volume combining each of a first series of serial dilutions of serum drawn from said animal and diluting each aliquot serially with equal volumes of with water to prepare a first and second series of serial volumetric dilutions of said serum;
- 10 b) Combining each dilution of a the first series of serial dilutions of said serum with a fixed concentration of a first, target chelate-fluorophore tracer composition comprising a metal-chelated reagent having the formula



15 wherein m is 0 or 1; n is 1, 2, or 3; R₁ and R₂ are p-CH₂C₆H₄-X-Y or H, wherein R₁ must be H when R₂ is p-CH₂C₆H₄-X-Y and R₂ must be H when R₁ is p-CH₂C₆H₄-X-Y; R₃ and R₄ are H, CH₃, or are fused into a cyclohexyl ring system; X is

20 -HNC(S)NH-, -NHC(O)- or -NH-C₃N₂Cl-NH-; Y is a fluorophore having a

Page 11 of 26

15 March, 2005

fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and of Claim 1,
wherein M is the target metal, and measuring the polarization of the fluorescent signal obtained when each resulting solution resulting from said combining is excited with
5 plane polarized light;

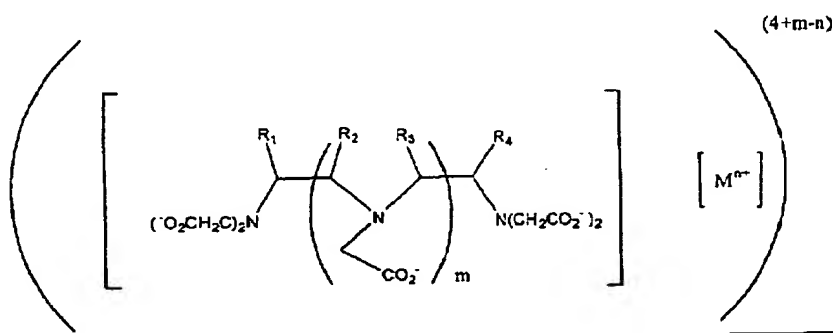
c) combining each dilution of a the second series of identical serial volumetric
dilutions of said serum with a second, chelate-fluorophore tracer composition
comprising a metal-chelated reagent having a formula identical to that of the chelate-
fluorophore tracer composition of step a) with respect to m, n, R₁, R₂, R₃, R₄, X, and
10 Y, but of Claim 1, wherein M is a non-target metal, said second tracer composition
being present at the same concentration as the first tracer composition, and measuring
the polarization of the fluorescent signal obtained when each resulting solution
resulting from said combining is excited with plane polarized light;

d) for each pair of solutions having the same extent of dilution, one solution that
15 results from the combining of step b) and the other solution that results from the
combining of step c), subtracting the measured value of the polarization of the
fluorescent signal produced by each the solution that is obtained by combining the
dilution of the second series of serial volumetric dilutions of said serum with
containing the non-target tracer composition of step c) b) from the measured value of
20 the polarization of the fluorescent signal produced by the corresponding solution that
is obtained by combining the dilution of the first series of serial volumetric dilutions
of said serum with the target tracer composition of step b) a) when measured at each
sample dilution, whereby a positive net value of said subtracting at any dilution less
than that producing a baseline signal for the target tracer composition indicates the

e) Repeating steps b) ~~and e)~~ through d) for as many non-target metals as may be required to fully define the metal selectivity of the polyclonal antibody according to its intended purpose.

- 10 a) taking two aliquots having an equal volume of said hybridoma supernatant or purified antibody preparation and diluting each aliquot serially with equal volumes of water to prepare a first and second series of serial volumetric dilutions of said hybridoma supernatant or purified antibody preparation;

- b) combining each dilution of a the first series of serial volumetric dilutions of said hybridoma supernatant or purified antibody preparation with a fixed concentration of a first, target chelate-fluorophore tracer composition comprising a metal-chelated reagent having the formula



Page 13 of 26

15 March, 2005

- wherein m is 0 or 1; n is 1, 2, or 3; R_1 and R_2 are $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ or H, wherein R_1 must be H when R_2 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ and R_2 must be H when R_1 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$; R_3 and R_4 are H, CH_3 , or are fused into a cyclohexyl ring system; X is -HNC(S)NH- , -NHC(O)- or $\text{-NH-C}_3\text{N}_3\text{Cl-NH-}$; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and of Claim 1, wherein M is the target metal, and measuring the polarization of the fluorescent signal obtained when each resulting solution resulting from said combining is excited with plane polarized light;
- 5 c) combining each dilution of a the second series of identical serial volumetric dilutions of said hybridoma supernatant or purified antibody preparation with a second, chelate-fluorophore tracer composition comprising a metal-chelated reagent having a formula identical to that of the fluorophore tracer composition of step b) with respect to m , R_1 , R_2 , R_3 , R_4 , X , and Y , but of Claim 1, wherein M is a non-target
- 10 metal, said second tracer composition being present at the same concentration as the first tracer composition, and measuring the polarization of the fluorescent signal obtained when each resulting solution resulting from said combining is excited with plane polarized light;
- 15 d) for each pair of solutions resulting from the combinings of steps b) and c), wherein the two solutions in the pair have the same extent of dilution, subtracting the measured value of the polarization of the fluorescent signal produced by each the solution that is obtained by combining the dilution of the second series of serial volumetric dilutions of the hybridoma supernatant or purified antibody preparation with containing the non-target tracer composition of step b) c) from the measured value of the polarization
- 20

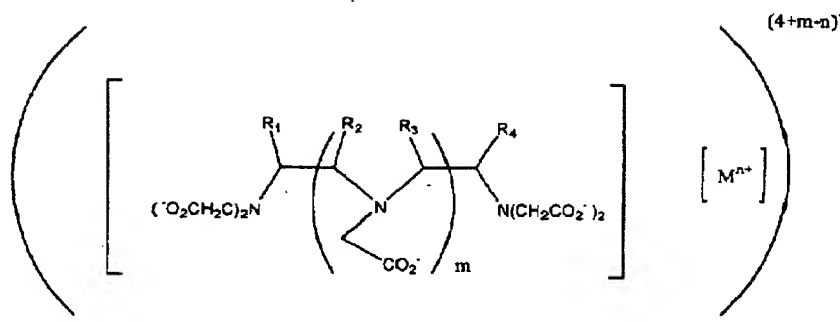
Page 14 of 26

15 March, 2005

- of the fluorescent signal produced by the solution that is obtained by combining the dilution of the first series of serial volumetric dilutions of the hybridoma supernatant or purified antibody preparation with the corresponding target tracer composition of step b) a) when measured at each sample dilution, whereby a positive net value from the subtracting at any dilution less than that producing a baseline signal for the target tracer composition indicates the presence of a monoclonal antibody that binds selectively to the target chelate-fluorophore composition and a zero or negative net value from the subtracting indicates no selectivity for said target chelate-fluorophore composition; and
- 10 e) repeating steps b) ~~and c)~~ through d) for as many non-target metals as may be required to fully define the metal selectivity of the monoclonal antibody according to its intended purpose.
12. (Amended Twice) An immunoassay method for determining the concentration of a target metal ion in an aqueous solution comprising:
- 15 a) combining an aliquot of said solution with a first assay reagent comprising a buffered solution of EDTA or DTPA to obtain a first resulting solution;
- b) adding to the first resulting solution a second assay reagent comprising the ~~corresponding~~ a target chelate-fluorophore tracer composition comprising a metal-chelated reagent having the formula
- 20

Page 15 of 26

15 March, 2005



wherein m is 0 if the first assay reagent is EDTA or 1 if the first assay reagent is DTPA; n is 1, 2, or 3; R_1 and R_2 are $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ or H, wherein R_1 must be H when R_2 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$ and R_2 must be H when R_1 is $p\text{-CH}_2\text{C}_6\text{H}_4\text{-X-Y}$; R_3 and R_4 are H, CH_3 , or are fused into a cyclohexyl ring system; X is -HNC(S)NH- , -NHC(O)- , or $\text{-NH-C}_3\text{N}_3\text{Cl-NH-}$; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and of Claim 1, wherein M is the target metal chelated thereto and is selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium, to obtain a second resulting solution;

c) adding to the second resulting solution a third assay reagent comprising a macromolecular biological binding agent that binds specifically to said target chelate-fluorophore tracer composition to obtain a third resulting solution;

d) measuring the polarization of the fluorescent signal obtained when the third resulting solution is excited with plane-polarized light; and

e) comparing this the polarization value of the fluorescent signal of step d) to these the

Page 17 of 26

15 March, 2005

~~wherein~~ M is the target metal to obtain a first resulting solution;

b) adding to the first resulting solution a second assay reagent comprising a macromolecular biological binding agent that binds specifically to said target chelate-fluorophore tracer composition to obtain a second resulting solution;

5 c) measuring the polarization of the fluorescent signal obtained when the second resulting solution is excited with plane-polarized light; and

d) comparing ~~this~~ the polarization value of the fluorescent signal of step c) to ~~these~~ the polarization values produced by a series of standard solutions, wherein each standard solution in said series comprises the first assay reagent of step a) and the second assay
10 reagent of step b), wherein M is the target metal and is present in a containing known concentrations ~~of said target metal.~~

14. (Original) The immunoassay method of Claim 12 or 13, wherein the aqueous solution is obtained by extraction of a solid sample, or a multiphasic sample that contains
15 solids, with one or more aqueous mineral acids.

15. (Original) The immunoassay method of Claim 12 or 13, wherein the aqueous solution is a water sample.

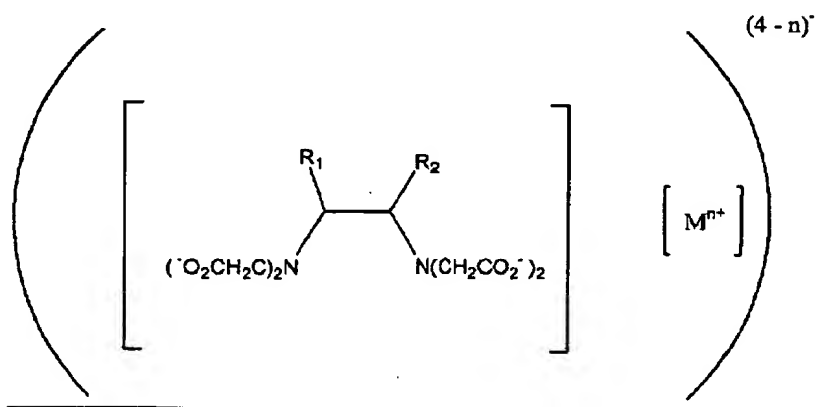
20 16. (Amended Twice) An immunoassay method for determining the concentration of lead in an aqueous extract of a solid sample; or of a multiphasic sample that contains solids, comprising:

a) combining an aliquot of said aqueous extract with a first assay reagent comprising a buffered solution of EDTA and ~~the corresponding~~ a target chelate-fluorophore tracer

Page 18 of 26

15 March, 2005

composition comprising a metal-chelated reagent having the formula



- 5 wherein n is 1, 2, or 3; R₁ and R₂ are p-CH₂C₆H₄-X-Y or H, wherein R₁ must be H
when R₂ is p-CH₂C₆H₄-X-Y and R₂ must be H when R₁ is p-CH₂C₆H₄-X-Y; R₃ and R₄
are H, CH₃, or are fused into a cyclohexyl ring system; X is -HNC(S)NH-, -NHC(O)-
or -NH-C₃N₃Cl-NH-; Y is a fluorophore having a fluorescence lifetime and quantum
yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by
10 fluorescence polarization; and of Claim 3, wherein M is lead to obtain a first resulting
solution;
- b) adding to the first resulting solution a second assay reagent comprising an aqueous
solution of a biological binding agent that binds specifically to said target chelate-
fluorophore tracer composition of step a) Claim 3, wherein M is lead, to obtain a
15 second resulting solution;
- c) measuring the polarization of the fluorescent signal obtained when the second
resulting solution is excited with plane-polarized light; and
- d) comparing the measured value of the polarization of the fluorescent signal of the
second resulting solution to a standard curve defined by the respective measured

Page 19 of 26

15 March, 2005

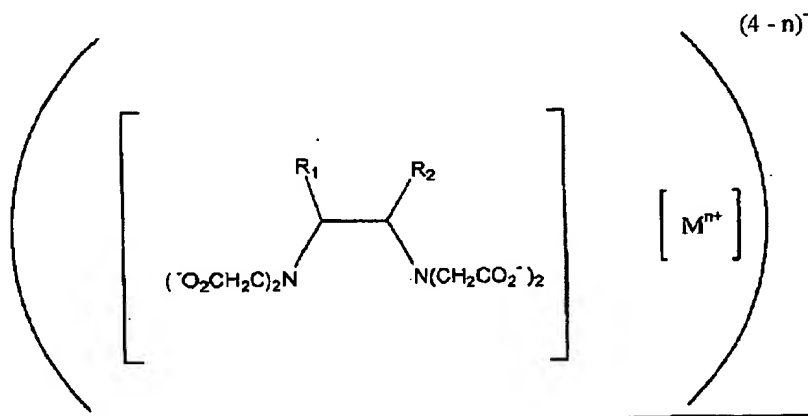
values of the polarization of the fluorescent signal produced by a series of standard solutions, wherein each standard solution in said series comprises the buffered solution of EDTA and the second assay reagent of step b), wherein the target metal is lead(II) and the lead is present in a containing known concentrations of lead(II).

5

17. (Amended Twice) An immunoassay method for determining the concentration of lead in a water sample, comprising:

a) combining an aliquot of said water sample with a first assay reagent comprising a buffered solution of EDTA and ~~the corresponding~~ a target chelate-fluorophore tracer composition comprising a metal-chelated reagent having the formula

10



wherein n is 1, 2, or 3; R₁ and R₂ are p-CH₂C₆H₄-X-Y or H, wherein R₁ must be H when R₂ is p-CH₂C₆H₄-X-Y and R₂ must be H when R₁ is p-CH₂C₆H₄-X-Y; R₃ and R₄ are H, CH₃, or are fused into a cyclohexyl ring system; X is -HNC(S)NH-, -NHC(O)- or -NH-C₃N₃Cl-NH-; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and ~~of Claim 3 wherein M is lead to obtain a first resulting~~

15

Page 20 of 26

15 March, 2005

solution;

b) adding to the first resulting solution a second assay reagent comprising a biological binding agent that binds specifically to said target chelate-fluorophore tracer composition to obtain a second resulting solution;

5 c) measuring the polarization of the fluorescent signal obtained when the second resulting solution is excited with plane-polarized light;

d) comparing the measured value of the polarization of the fluorescent signal of the second resulting solution to a standard curve defined by the values of the polarization of the fluorescent signal produced by a series of standard solutions, wherein each standard solution in said series comprises the buffered solution of EDTA and the second assay reagent of step b), wherein the target metal is lead(II) and the lead is present in a containing known concentrations of lead(II); and

e) assigning a value to the concentration of lead in the water sample.

15 18. (Original) The immunoassay method of Claim 16 or 17, further comprising an assay diluent comprising between about 10 - 100 mM sodium bicarbonate or HEPES, between about 10-100 μ M EDTA, and between about 1- 10 nM lead chelate-fluorophore tracer composition of Claim 1 wherein M is Pb, n is 2, R₁ is p -CH₂C₆H₄-X-Y, R₂ is H; X is -HNC(S)NH-; and Y is fluorescein.

20

19. (Original) The immunoassay method of Claim 16 or 17, further comprising an antibody comprising the rabbit polyclonal antiserum raised against the EDTA-Pb complex and screened for cross-reactivity with aluminum, iron, chromium, zinc, copper, and nickel.

Page 21 of 26

15 March, 2005

20. (Cancelled) A test kit for measuring the concentration of a target metal in a test sample, comprising:
- a) at least one standard solution containing a known concentration of the target metal;
 - b) a first assay reagent comprising a base, a chelating agent, and the corresponding target metal chelate-fluorophore tracer composition of Claim 1 wherein M is the target metal; and
 - c) a second assay reagent containing a known concentration of the biological binding agent responsive to the target metal chelate-fluorophore tracer composition.
- 10 21. (Cancelled) A test kit for measuring the concentration of lead in a test sample, comprising:
- a) at least one standard solution containing a known concentration of lead(II);
 - b) a first assay reagent comprising a base, EDTA, and the corresponding lead chelate-fluorophore tracer composition of Claim 3 wherein n is 1, Y is fluorescein, and M is Pb;
 - c) a second assay reagent containing a known concentration of the biological binding agent responsive to the lead chelate-fluorophore tracer composition; and
 - d) optionally, an extraction fluid suitable for obtaining an aqueous extract of a solid sample, or a multiphasic sample that contains solids.